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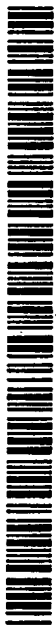
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(54) Title: EXERCISE AND MUSCLE ENHANCEMENT FORMULATIONS

(57) Abstract: Disclosed are exercise and muscle enhancing formulations of creatine with an insulin modulating agent such as arginine. Also included are precursors, biological equivalents, or intermediate products of arginine such as L-citrulline, L-ornithine, L-glutamine, or L-lysine, their salts, esters, peptides, dipeptides or complexes with other chemicals combined with creatine. The creatine and insulin modulating substance such as L-arginine are preferably in sustained-release form so as to modulate and facilitate the absorption and transport of the creatine to muscle via increased vasodilation. The sustained-release combination of creatine monohydrate and L-arginine free base prolong the time period for muscle perfusion and enhance the supply of creatine to the muscles especially during extended athletic activity or body building. The arginine or biological equivalent, due to its endogenous anti-oxidant capacity via nitric oxide also serves to preserve creatine kinase which is oxidized by free radicals. Creatine kinase is responsible for converting creatine to phosphocreatine which is stored and recycled in muscles.

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EXERCISE AND MUSCLE ENHANCEMENT FORMULATIONS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to immediate-release and sustained-release compositions containing substrates for the production of nitric oxide, or insulin mediating substances in combination with creatine. More particularly, sustained-release compositions containing arginine, L-citrulline, L-ornithine, L-lysine, L-glutamine, and their salts, esters, complexes, dipeptides, peptides, as well as botanical substances and extracts that serve as substrates for the production of nitric oxide or insulin mediation are combined with creatine, either in sustained-release or immediate-release form, to enhance the activity of creatine in skeletal muscle. The compositions are useful for enhancing athletic endurance, and body building, and prolong and increase the activity and supply of creatine to and in muscle tissue.

The Nitric Oxide/Insulin Modulating Component

Substances generated by cells within the vessel wall have been demonstrated to be important regulators of smooth muscle tone. The vascular endothelium is responsible for the generation of these vasoactive compounds. Endothelium-derived relaxing factor (EDRF) was initially believed to be the agent responsible for mediating or regulating vasodilation. S. Moncada was one of the first to identify that EDRF was nitric oxide and that the biosynthetic precursor to nitric oxide was the amino acid L-arginine (Palmer RMJ, Ferrige AG, Moncada S, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524-526), (Palmer RMJ, Ashton DS, Moncada S, Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature

1988;333:664-666), (Palmer RMJ, Rees DD, Ashton DS, Moncada S, L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun* 1988;153:1251-1256)

It is now widely known that Nitric oxide (NO) plays an important role in the regulation of many physiological functions such as vasodilatation, atherosclerosis, platelet aggregation, restenosis, hypertension, reperfusion injury, renal failure, and erectile dysfunction (Ignarro LJ. *Physiological Significance of Endogenous Nitric Oxide. Seminars in Perinatology*, 1991; Vol. 15, 1; 20-26). Endogenous NO is synthesized by different isoforms of the enzyme nitric oxide synthase (NOS) from the amino acid L-arginine. (Moncada S, Higgs EA. *The L-arginine-nitric oxide pathway (N England J Med* 1993; 329:2002-2012). NOS is a cytochrome p450 protein enzyme which requires certain cofactors. The biosynthesis of endogenous NO from L-arginine by NOS involves the basic guanidino nitrogen atoms of L-arginine, and the intermediate product is L-citrulline.

Creatine, or methyl guanidine-acetic acid is manufactured by the body to supply energy to the muscles. One of the endogenous precursors for the production of creatine is L-arginine. Creatine can be made by the body (endogenous) or be supplied by the diet or through supplementation (exogenous). The liver is responsible for the majority of the endogenous biosynthesis of creatine within the body, as well as the metabolism of exogenous creatine from supplemental dietary creatine or L-arginine.

The liver contains enzymes that convert drugs and other dietary chemicals to metabolites which can then be more easily eliminated by the body in the urine and the feces. This conversion process or biotransformation of the drug or therapeutic compound may, in many cases, influence the duration of action or the intensity (pharmacodynamics) of the compound. The rate of metabolism and the extent of metabolism can have a

profound effect on the therapeutic parameters of the drug, which in turn is a reflection of the bioavailability.

Because of metabolism issues, many drugs or natural therapeutic agents must be taken numerous times a day to achieve the desired pharmacological effects.

Cytochrome p450 is one of the many pharmaceutical-metabolizing enzyme systems of the liver, but is perhaps the enzyme system that plays the most important role in determining the rate of elimination of drugs. Each of the various enzyme systems in the liver is comprised of many individual enzymes, each of which is capable of metabolizing a wide variety of therapeutic substances or chemicals. The cytochrome P450 system in the liver consists of at least ten individual P450 enzymes. The metabolism of therapeutic agents by cytochrome P450 often represents the rate-limiting step in pharmaceutical elimination. Therefore, factors that decrease the activity of P450 enzymes usually prolong the effects of drugs, whereas factors that increase cytochrome P450 activity have the opposite effect.

Since the conversion of L-arginine to NO is a metabolic process involving a cytochrome P450 enzyme called NOS or nitric oxide synthase (Sessa WC, The nitric oxide synthase family of proteins; J Vasc Res 1994; 31:131-143), the effects of slowing the rate of presentation of L-arginine to the liver, the gastrointestinal tract, and the endothelium, and its conversion to NO via NOS metabolism is unknown. Cytochrome P450 or NOS enzymes are also located in the gastrointestinal tract, but the effects produced by slowing down the rate of presentation and exposure of the drug or therapeutic agent to these enzymes is uncertain. Therapeutic agents that are subject to first pass metabolism via the portal vein, and are presented to the liver prior to systemic circulation, may be influenced by incorporation in sustained-release dosage forms, but the effect on metabolism may be different for each compound, and the production or ratio of metabolites is unpredictable.

L-arginine, L-citrulline, L-ornithine, L-glutamine, arginine silicate, arginine aspartate, their salts, esters, complexes, and dipeptides or polypeptides are preferred substrates for the endogenous production of NO or conversion to its substrate, L-arginine. Unfortunately, fairly large doses (5 to 30 grams per dose) of L-arginine are required to enhance NO production or insulin mediated vasodilation. Surprisingly, the inventor has found that a single oral dose of immediate-release L-arginine in excess of 4 grams results in rapid onset of diarrhea, particularly if the dose is given as L-arginine free base. Dose dumping is likely to result in less absorption of single doses in excess of a few grams and loss of substrate for endothelial production of NO, making oral supplementation with L-arginine an unacceptable route of administration. Furthermore, saturation of absorption systems is likely. L-arginine free base, which gram for gram yields the most arginine for substrate production of NO, has a pH range of 10.5-12.0, and is extremely alkaline, yet contains about 18% more actual L-arginine than the hydrochloride salt. Since many grams of exogenous L-arginine need to be consumed per day to derive a cardiovascular benefit, the free base would be the preferred form. In a pilot study conducted by the inventor in three human subjects, oral consumption of a single dose of 4 grams of L-arginine free base in water resulted in bowel intolerance within a few hours in all three subjects. After a two week washout, the same subjects were given the same dose of 4 grams in water again with the same results. Diarrhea generally manifests as intestinal hypermotility and rapid transport, speeding up gastric emptying and shortening transit time for solutes in the window of absorption.

Surprisingly, when the same subjects were given sustained-release formulations of L-arginine, greater absorption was possible due to reduced bowel intolerance. In addition, the saturation or overwhelming of absorption systems was avoided. In this way less L-arginine is lost to diarrhea, and more is absorbed for vasodilation or production of nitric oxide.

The majority of the studies conducted with L-arginine that relate to the benefits of NO production or insulin mediated vasodilation have either involved intravenous administration or oral administration of immediate-release formulations in repeated doses throughout the day. For example, in the study "Effect of Supplemental Oral L-Arginine on Exercise Capacity in Patients With Stable Angina Pectoris" by Ceremuzynski et al; American Journal of Cardiology; 1997,80 (3); 331-3, the subjects were given two 1 gram capsules (2 grams) 3 times a day, at 9 A.M., 2 P.M., and 10 P.M.. An example of the intravenous administration of L-arginine can be found in "L-Arginine Infusion Decreases Platelet Aggregation Through An Intraplatelet Nitric Oxide Release"; Marietta et al; Thrombosis Research; 1997; 88, (2): 229-35. In that study subjects were given 30 grams of L-arginine as an infusion. This raised circulating levels of L-arginine up to 100 fold compared to baseline levels. This same dose would have been impossible to administer orally as it would not be tolerated by the gastrointestinal tract.

In one study, the average bioavailability of oral L-arginine during chronic administration for 12 weeks was only 40-50% (Tangphao, O, et al, Clinical Science; 1999: 96, 199-207). In another pharmacokinetic study, the elimination half-life of a 6 gram oral dose was about 79.5 minutes, or 1 hour and 20 minutes (Bode-Boger, S., et al, Br J Clin Pharmacol 1998; 46: 489-497). The time to reach maximum or peak plasma concentration (Tmax) was 90 minutes. The onset and duration of the pharmacodynamic effects (vasodilation and production of endogenous nitric oxide) closely corresponded to the plasma concentration half-life of L-arginine, as indicated by the equilibration half-life of approximately 6 minutes between plasma concentration and effect in pharmacokinetic-pharmacodynamic analysis. Therefore, the vascular effects of L-arginine are closely correlated with its plasma concentrations. In rats, injected arginine is rapidly absorbed from the gastrointestinal tract into the blood stream and is quickly taken up by the tissues and is no longer present after about 2 hours. The plasma half life was approximately 1 hour (Noeh FM, et al, Life Sci 1996; 58 (8):

PL131-8). Therefore, there is a need to prolong the supply of arginine for production of beneficial effects.

Likewise, infusions of both L and D forms of arginine have been shown to increase dilation in the peripheral vascular but only at high doses ("Dilator Actions of Arginine in Human Peripheral Vasculature"; Calver, A, et al; Clin Sci (Colch) 1991, 81(5):695-700. In this study, there was no response when doses of 10 and 40 $\mu\text{mol}/\text{min}$ were administered, however, at doses of 160 $\mu\text{mol}/\text{min}$, both L and D arginine free base and the hydrochloride salt cause a significant increase in forearm blood flow compared with controls. This and other evidence supports the idea that arginine may act as a vasodilator in a way that is not presently understood, or by stimulating insulin release. D-arginine is not a substrate for nitric oxide synthase (NOS) yet at higher doses it produced an effect on vasodilation.

L-arginine is able to stimulate insulin release, but at higher doses (Blachier, F, et al: 1989, Endocrinology; 124:131-41). Insulin is known to produce vasodilation and an approximate two fold increase in resting skeletal muscle blood flow in insulin sensitive humans. This effect has also been demonstrated in normal healthy individuals. Insulin's effect in increasing skeletal muscle perfusion is highly correlated with its ability to stimulate glucose uptake. Skeletal muscle blood flow and glucose uptake can be stimulated with steady state insulin concentrations in the physiologic to subpharmacologic range (Baron, A, et al, "Insulin-mediated Skeletal Muscle Vasodilation Contributes to Both Insulin Sensitivity and Responsiveness in Lean Humans" J. Clin. Invest. 1995. 96:786-792).

Intravenous administration remains undesirable because of the expense and difficulty involved in administering such medications intravenously. Subjects will always prefer oral administration over injection or infusion, as it avoids painful insertion of needles. Additionally, there is the enhanced danger of infection. Intravenous administration also involves a

clinic and a medical professional, and is not suitable or practical for daily usage.

Oral administration, while desirable, represents problems in that administration of the compound in conventional oral dosage forms at levels necessary to generate nitric oxide or stimulate insulin release results in diarrhea, thus significantly reducing the bioavailability of the compound. Consequently, despite the usefulness of L-arginine and its biological equivalents in treating a variety of medical conditions, there remains no good dosage form for administering L-arginine in the quantities necessary for generation of significant pharmacological amounts of nitric oxide. There is therefore a need for improved dosage forms of L-arginine and other precursors of vasoactive compounds and their biological equivalents for use in oral administration.

The Creatine Component

After being made in the liver, creatine is transported by the blood and taken up by muscle cells where it is converted into creatine phosphate, otherwise known as phosphocreatine. The enzyme responsible for this conversion is creatine kinase which is found inside muscle cells. Creatine kinase is also found in the liver, and is an enzyme especially susceptible to oxidative degradation. As creatine cycles back and forth between creatine and phosphocreatine, it produces energy to the muscle cells, but only briefly. This immediate burst of energy has an extremely short half life of about 15 seconds. Dietary supplementation with large amounts of creatine, or creatine loading, has been one way of attempting to overcome the quick exhaustion of creatine stores when there is intense and prolonged activation of muscles during athletic activity or weight training. It is believed that by loading the muscles with extra creatine, more creatine would be available for energy production by the muscles after the initial exhaustion of endogenous creatine. But the amount of creatine present in muscle cells can saturate the sodium transport system responsible for enabling more creatine

to enter the muscles, reducing the flow of new creatine as already present creatine stores are blocking the diffusion gradient. Consuming more immediate-release creatine does not necessarily push more creatine into the muscles because it can shut down the sodium pump responsible for shuttling the creatine into muscle to begin with. In addition, a high creatine concentration will downregulate muscle creatine transport (Proc. Natl. Acad. Sci. USA 85:807-811, 1988).

Creatine accumulation can be substantially increased in human skeletal muscle when ingested with large quantities of simple carbohydrates, but the amounts of carbohydrates necessary are far too high to be physiologically acceptable for repeated use. For example, a 94 gram dose of carbohydrates in the form of glucose and simple sugars was needed with each 5 gram dose of creatine to increase muscle creatine by about 25%. (Am J. Physiol. 271 (Endocrinol. Metab. 34) E821-E826, 1996). This effect is believed to be related to carbohydrate-mediated insulin release, which presumably would stimulate sodium-dependent muscle creatine transport. Insulin has been demonstrated to stimulate muscle blood flow, and insulin mediated vasodilation, or perfusion, is driven by endothelium-derived nitric oxide. However, nitric oxide may only act as a mediator for this process.

The rate of muscle perfusion or vasodilation is an important determinant of the overall rate of glucose uptake. The rate of infusion of insulin effects the transport and storage of creatine, but the enhancement of creatine transport and storage by arginine or a biological nitric oxide or insulin mediated equivalent substance is unexpected.

Surprisingly, the inventor has found that by incorporating arginine or a biological equivalent (for example L-citrulline) with creatine, muscle creatine stores may be increased. An even more effective formulation for accomplishing creatine muscle transport and accumulation is the combination of sustained-release arginine with sustained-release creatine. Sustained-release arginine is more effective because more arginine substrate

is made available through better absorption in the gastrointestinal tract. Therefore, more substrate is available for endothelial production of nitric oxide mediated insulin, which stimulates better creatine transport to and accumulation in muscle. Furthermore, by coupling the slow presentation of both substances simultaneously, a type of nitric oxide shuttle for more effective delivery of creatine to muscle is provided.

By slowing down the rate of presentation of the creatine to the liver and the muscles, especially during intense exercise or body building workouts, the need for normal creatine loading, which is inefficient, is avoided. Instead, the supply of creatine is constant, and is not working against a concentration gradient for entry to muscle. The slow, long term supply of creatine, which spans many hours of exercise activity, provides a metered injection of creatine as it is exhausted from muscle stores. This type of system is more effective during intense muscular activity than during sedentary periods because of the increased catabolism of creatine to creatinine.

By slowing down the rate of increased substrate availability of a nitric oxide and insulin mediating substance such as arginine or a biological equivalent, prolonged vasodilation can be achieved. Instead of a sudden burst of nitric oxide and concomitant decay, long term conversion to NO or stimulation of insulin release can occur. Likewise, long term vasodilatory effects from insulin and a sustained increase in blood supply drives creatine and other nutrient and energy rich co-factor availability to skeletal muscle. By increasing the supply and controlling the rate of blood flow to muscle, the rate of glucose uptake can be effected.

Nitric oxide also functions as an anti-oxidant by suppressing the production of superoxide anion, and thereby limits peroxynitrate production. Peroxynitrate is a powerful free radical, capable of producing significant damage on a cellular level, and particularly in the endothelium. At suboptimal L-arginine concentrations, nitric oxide synthase (NOS), the

enzyme responsible for conversion of L-arginine to nitric oxide, is capable of making damaging free radical species as conversion is rapidly shifted to superoxide. By increasing intracellular levels of L-arginine, the direction of conversion of substrate can be shifted from reactive oxygen species such as superoxide, peroxynitrate, and hydrogen peroxide in the direction of free radical quenching nitric oxide synthesis. This will overwhelm the potential for turning nitric oxide into a damaging substance.

Intense exercise increases production of reactive oxygen species (free radicals). Athletes have a real need for an anti-oxidant system that has direct effects on the endothelium. Creatine kinase, the enzyme responsible for conversion of creatine to phosphocreatine, is oxidized by free radicals. The anti-oxidant properties of nitric oxide enhancement via increased supply of exogenous L-arginine, or the intermediate product, L-citrulline, should serve to extend and prolong the integrity of creatine kinase, and thereby facilitate the cycling of creatine to phosphocreatine. This should enhance creatine stores in myocytes, and provide a better environment for the entire process.

The maximum oxygen uptake or VO_2 max, an index for aerobic capacity, is partly determined by the availability and control of blood flow to the active muscles. Aerobic capacity can be increased or reduced by vascular reactivity.

Therefor, prolonged vasodilation, coupled with anti-oxidant production via nitric oxide to suppress superoxide anion production is desirable. Exercise stimulates glucose transport utilization, and chronic endurance training upregulates type I and type III nitric oxide synthetase (NOS) isoforms in soleus muscle. The fact that exercise stimulates the protein expression of the enzyme responsible for producing nitric oxide is evidence of the importance of the L-arginine/ NOS/nitric oxide pathway in body building and athletic training and performance. Nitric oxide is probably playing a role in modulating contractile function.

The preferred insulin mediating supplement to be coadministered with creatine is L-arginine free base. In a preferable embodiment, the amount of L-arginine free base in a single oral dose ranges from about 1 gram to about 10 grams, and the amount of creatine monohydrate ranges from about 500 mg. to 10 grams. In a more preferable embodiment, the amount of L-arginine free base in a single oral dose ranges from about 3 grams to 8 grams and the amount of creatine monohydrate ranges from about 2 grams to 8 grams. In a still more preferable embodiment, the amount of L-arginine free base in a single oral dose would be about 5 grams and the amount of creatine monohydrate in a single oral dose would be about 5 grams.

The preferred formulation of each substance for coadministration would be sustained-release. The same dosage levels of each ingredient that were specified above would be applicable in the sustained-release form, but higher doses of L-arginine in particular could be administered. Controlled release within the scope of this invention can be taken to mean any one of a number of extended release dosage forms. The following terms may be considered to be substantially equivalent to controlled release, for the purposes of the present invention: continuous release, controlled release, delayed release, depot, gradual release, long-term release, programmed release, prolonged release, proportionate release, protracted release, repository, retard, slow release, spaced release, sustained release, time coat, timed release, delayed action, extended action, layered-time action, long acting, prolonged action, repeated action, slowing acting, sustained action, sustained-action medications, and extended release. Further discussions of these terms may be found in Lesczek Krowczynski, Extended-Release Dosage Forms, 1987 (CRC Press, Inc.).

The various controlled release technologies cover a very broad spectrum of drug dosage forms. Controlled release technologies include, but are not limited to physical systems and chemical systems. Physical systems include, but not limited to, reservoir systems with rate-controlling membranes, such as microencapsulation, macroencapsulation, and

membrane systems; reservoir systems without rate-controlling membranes, such as hollow fibers, ultra microporous cellulose triacetate, and porous polymeric substrates and foams; monolithic systems, including those systems physically dissolved in non-porous, polymeric, or elastomeric matrices (e.g., non-erodible, erodible, environmental agent ingress, and degradable), and materials physically dispersed in non-porous, polymeric, or elastomeric matrices (e.g., non-erodible, erodible, environmental agent ingress, and degradable); laminated structures, including reservoir layers chemically similar or dissimilar to outer control layers; and other physical methods, such as osmotic pumps, or adsorption onto ion-exchange resins.

Chemical systems include, but are not limited to, chemical erosion of polymer matrices (e.g., heterogeneous, or homogeneous erosion), or biological erosion of a polymer matrix (e.g., heterogeneous, or homogeneous).

Hydrogels may also be employed as described in "Controlled Release Systems: Fabrication Technology", Vol. II, Chapter 3; p 41-60; "Gels For Drug Delivery", Edited By Hsieh, D.

While a preferable mode of sustained-release drug delivery will be oral, other modes of delivery of sustained-release compositions according to this invention may be used. These include mucosal delivery, nasal delivery, ocular delivery, transdermal delivery, parenteral controlled release delivery, vaginal delivery, rectal delivery, and intrauterine delivery.

There are a number of sustained-release drug formulations that are developed preferably for oral administration. These include, but are not limited to, microencapsulated powders, osmotic pressure-controlled gastrointestinal delivery systems; hydrodynamic pressure-controlled gastrointestinal delivery systems; membrane permeation-controlled gastrointestinal delivery systems, which include microporous membrane permeation-controlled gastrointestinal delivery devices; gel diffusion-

controlled gastrointestinal delivery systems; and ion-exchange-controlled gastrointestinal delivery systems, which include cationic and anionic drugs. The preferred sustained-release system is an oil microencapsulated sustained-release powder dosage form that can be mixed with liquid and consumed as a drink mix beverage.

Combinations of coating agents may also be incorporated such as ethylcellulose and hydroxypropylmethylcellulose, which can be mixed together and sprayed onto the L-arginine in a fluid bed granulator. Another method employs mixtures of a high temperature melting vegetable oil with an iodine value maximum of about 5 and a melting point of about 145°F with a cellulose ether such as ethylcellulose. This combination can be processed in a vertical or horizontal high intensity mixer or a blender that is jacketed so as to allow a hot water bath to circulate around the mixer to elevate the temperature of the oil to the melting point. The L-arginine and creatine powder are then mixed with the molten oil until complete coverage is achieved (about 5-10 minutes), cooled, and the ethylcellulose sprayed onto the particles. The finished product is a microencapsulated, free-flowing sustained-release powder with an extended release profile.

Aqueous dispersions may also be formulated. Of particular interest for L-arginine aqueous dispersions are polymeric hydroabsorptive agents such as hydrocolloid fibers, which will help to absorb water in the gastrointestinal tract, helping to minimize the potential for diarrhea, while also providing some sustained-release effects.

Examples of carriers useful in solid and aqueous dispersions according to the invention include, but are not limited to, water-soluble polymers such as guar gum, glucomannan, psyllium, gum acacia, polyethylene glycol, polyvinylpyrrolidone, hydroxypropyl methylcellulose, and other cellulose ethers such as methylcellulose, and sodium carboxymethylcellulose. Powdered drink mixes which are designed to be added to water or other liquids incorporating microspheres of sustained-

release L-arginine, coated with a high melting point vegetable oil, and then mixed with a hydrocolloid polymer such as those previously listed are also suitable.

Furthermore, compositions of L-arginine or biological equivalents and creatine according to the invention may be administered or coadministered with conventional pharmaceutical binders, excipients and additives. Many of these are controlled-release polymers which must be used in sufficient quantities to produce a sustained-release effect. The use of low levels of these ingredients will not result in sustained-release when they are used as a diluent, binder, or disintegrant. These include, but are not limited to, gelatin, natural sugars such as raw sugar or lactose, lecithin, mucilage, plant gums, pectin's or pectin derivatives, algal polysaccharides, glucomannan, agar and lignin, guar gum, locust bean gum, acacia gum, xanthan gum, carrageenan gum, karaya gum, tragacanth gum, ghatti gum, starches (for example corn starch or amylose), dextran, polyvinyl pyrrolidone, polyvinyl acetate, gum arabic, alginic acid, tylose, talcum, lycopodium, silica gel (for example colloidal), cellulose and cellulose derivatives (for example cellulose ethers, cellulose ethers in which the cellulose hydroxy groups are partially etherified with lower saturated aliphatic alcohols and/or lower saturated, aliphatic oxyalcohols, for example methyl oxypropyl cellulose, methyl cellulose, hydroxypropyl methyl cellulose, hydroxypropyl methyl cellulose phthalate, cross-linked sodium carboxymethylcellulose, cross-linked hydroxypropylcellulose, high-molecular weight hydroxymethylpropylcellulose, carboxymethyl-cellulose, low-molecular weight hydroxypropylmethylcellulose medium-viscosity hydroxypropylmethylcellulose hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, alkylcelluloses, ethyl cellulose, cellulose acetate, cellulose propionate (lower, medium or higher molecular weight), cellulose acetate propionate, cellulose acetate butyrate, cellulose triacetate, methyl cellulose, hydroxypropyl cellulose, or hydroxypropylmethyl cellulose), fatty acids as well as magnesium, calcium or aluminum salts of fatty acids with

12 to 22 carbon atoms, in particular saturated (for example stearates such as magnesium stearate), polycarboxylic acids, emulsifiers, oils and fats, in particular vegetable (for example, peanut oil, castor oil, olive oil, sesame oil, cottonseed oil, corn oil, wheat germ oil, sunflower seed oil, cod liver oil, in each case also optionally hydrated); glycerol esters and polyglycerol esters of saturated fatty acids $C_{12}H_{24}O_2$ to $C_{18}H_{36}O_2$ and their mixtures, it being possible for the glycerol hydroxy groups to be totally or also only partly esterified (for example mono-, di- and triglycerides); high melting point hydrogenated vegetable oils suitable for microencapsulation; pharmaceutically acceptable mono- or multivalent alcohols and polyglycols such as polyethylene glycol and derivatives thereof, esters of aliphatic saturated or unsaturated fatty acids (2 to 22 carbon atoms, in particular 10-18 carbon atoms) with monovalent aliphatic alcohols (1 to 20 carbon atoms) or multivalent alcohols such as glycols, glycerol, diethylene glycol, pentacrythritol, sorbitol, mannitol and the like, which may optionally also be etherified, esters of citric acid with primary alcohols, acetic acid, urea, benzyl benzoate, dioxolanes, glycerofomals, tetrahydrofurfuryl alcohol, polyglycol ethers with C_1 - C_{12} -alcohols, dimethylacetamide, lactamides, lactates, ethylcarbonates, silicones (in particular medium-viscous polydimethyl siloxanes), calcium carbonate, sodium carbonate, calcium phosphate, sodium phosphate, magnesium carbonate and the like.

Other substances that may be used include: cross-linked polyvinyl pyrrolidone, carboxymethylamide, potassium methacrylatedivinylbenzene copolymer, high-molecular weight polyvinylalcohols, low-molecular weight polyvinylalcohols, medium-viscosity polyvinylalcohols, polyoxyethyleneglycols, non-cross linked polyvinylpyrrolidone, polyethylene glycol, sodium alginate, galactomannone, carboxypolymethylene, sodium carboxymethyl starch, sodium carboxymethyl cellulose or microcrystalline cellulose; polymerizates as well as copolymerizates of acrylic acid and/or methacrylic acid and/or their esters, such as, but not limited to poly(methyl methacrylate), poly(ethyl

methacrylate), poly(butyl methacrylate), poly (isobutyl methacrylate), poly(hexyl methacrylate), poly (isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), or poly(octadecyl acrylate); copolymerizates of acrylic and methacrylic acid esters with a lower ammonium group content (for example Eudragit® RS, available from Rohm, Somerset, NJ), copolymerizates of acrylic and methacrylic acid esters and trimethyl ammonium methacrylate (for example Eudragit® RL, available from Rohm, Somerset, NJ); polyvinyl acetate; fats, oils, waxes, fatty alcohols; hydroxypropyl methyl cellulose phthalate or acetate succinate; cellulose acetate phthalate, starch acetate phthalate as well as polyvinyl acetate phthalate, carboxy methyl cellulose; methyl cellulose phthalate, methyl cellulose succinate, -phthalate succinate as well as methyl cellulose phthalic acid half ester; zein; ethyl cellulose as well as ethyl cellulose succinate; shellac, gluten; ethylcarboxyethyl cellulose; ethylacrylate-maleic acid anhydride copolymer; maleic acid anhydride-vinyl methyl ether copolymer; styrol-maleic acid copolymerizate; 2-ethyl-hexyl-acrylate maleic acid anhydride; crotonic acid-vinyl acetate copolymer; glutaminic acid/glutamic acid ester copolymer; carboxymethylethylcellulose glycerol monoctanoate; cellulose acetate succinate; polyarginine; poly (ethylene), poly (ethylene) low density, poly (ethylene) high density, poly (propylene), poly (ethylene oxide), poly (ethylene terephthalate), poly (vinyl isobutyl ether), poly (vinyl chloride) or polyurethane. Mixtures of any of the substances or materials listed herein may also be used in the practice of the invention.

Plasticizing agents that may be considered as coating substances useful are: Citric and tartaric acid esters (acetyl-triethyl citrate, acetyl tributyl-, tributyl-, triethyl-citrate); glycerol and glycerol esters (glycerol diacetate, - triacetate, acetylated monoglycerides, castor oil); phthalic acid esters (dibutyl-, diamyl-, diethyl-, dimethyl-, dipropyl-phthalate), di-(2-methoxy- or 2-ethoxyethyl)-phthalate, ethylphthalyl glycolate, butylphthalylethyl glycolate and butylglycolate; alcohols (propylene glycol,

polyethylene glycol of various chain lengths), adipates (diethyladipate, di-(2-methoxy- or 2-ethoxyethyl)-adipate; benzophenone; diethyl- and diburylsebacate, dibutylsuccinate, dibutyltartrate; diethylene glycol dipropionate; ethyleneglycol diacetate, -dibutyrate, -dipropionate; tributyl phosphate, tributyrin; polyethylene glycol sorbitan monooleate (polysorbates such as Polysorbar 50); sorbitan monooleate.

L-arginine or equivalent and creatine according to the invention may be orally administered or coadministered in a liquid dosage form. For the preparation of solutions or suspensions it is, for example, possible to use water or physiologically acceptable organic solvents, such as alcohols (ethanol, propanol, isopropanol, 1,2-propylene glycol, polyglycols and their derivatives, fatty alcohols, partial esters of glycerol), oils (for example peanut oil, olive oil, sesame oil, almond oil, sunflower oil, soya bean oil, castor oil, bovine hoof oil), paraffins, dimethyl sulphoxide, triglycerides and the like.

In the case of drinkable solutions the following substances may be used as stabilizers or solubilizers: lower aliphatic mono- and multivalent alcohols with 2-4 carbon atoms, such as ethanol, n-propanol, glycerol, polyethylene glycols with molecular weights between 200-600 (for example 1 to 40% aqueous solution), gum acacia, guar gum, or other suspension agents selected from the hydrocolloids may also be used.

It is also possible to add preservatives, stabilizers, buffer substances, flavor correcting agents, sweeteners, colorants, antioxidants and complex formers and the like. Complex formers which may be for example be considered are: chelate formers such as ethylene diamine retranscetic acid, nitrilotriacetic acid, diethylene triamine pentacetic acid and their salts.

Furthermore, sustained-release L-arginine/creatine according to the invention may be administered separately, or may coadministered with other inventive controlled release biological equivalents or other therapeutic

agents. Coadministration in the context of this invention is defined to mean the administration of more than the two therapeutic agents in the course of a coordinated treatment to achieve an improved clinical outcome. Such coadministration may also be coextensive, that is, occurring during overlapping periods of time.

It may be preferable at times to administer the creatine in immediate-release form and the arginine in sustained-release form, or to mix some immediate-release creatine with sustained-release creatine and sustained-release arginine to get an initial loading dose of creatine into the system.

The combination of creatine and arginine may be used as a food additive or incorporated into a candy bar or other confection like delivery system or food.

Preferred concurrently administered compounds would be selected from the anti-oxidants, and may include; vitamin E, selenium, beta carotene, vitamin C, α -lipoic acid, tocotrienols, N-acetylcysteine, co-enzyme Q-10, Pycnogenol® (French maritime pine bark extract, Henkel, Inc.), extracts of rosemary such as carnosol, botanical anti-oxidants such as green tea polyphenols, grape seed extract, cox-1 type inhibitors such as resveratrol, ginkgo biloba, and garlic extracts. Other amino acids such as L-cysteine or L-citrulline may be added. Combination with an acetylcholine precursor such as choline chloride or phosphatidylcholine may be desirable to enhance vasodilation.

If an ester or prodrug of either substance is employed, the following could be acceptable; alkyl, ethyl, methyl, propyl, isopropyl, butyl, isobutyl, or t-butyl esters.

If a salt is employed, it could be selected from the following; hydrochloride, glutamate, aspartate, butyrate, or glycolate.

Examples:**Example 1:**

Equal amounts of creatine monohydrate and L-arginine hydrochloride are blended together and delivered as a powder in a sachet or packet to be mixed in water. Each pre-measured dose contained 5 grams of creatine monohydrate and 2 grams of L-arginine hydrochloride. Athletes were instructed to consume one packet mixed in water three times per day, for a total daily dose of 6 grams of L-arginine and 15 grams of creatine. After 3 weeks of supplementation according to this program, athletes will experience increases in stamina and endurance and increase muscle mass when compared with the same dose of creatine without L-arginine.

Example 2:

Creatine monohydrate and L-arginine free base are added in equal amounts to a Littleford W-10 verticle high intensity mixer which is capable of operating at high temperatures. The unit was fitted with a tower-mounted, hydraulic atomizing nozzle with heated tanks and heated/insulated lines to enable hot oil to be applied at high temperatures. A hydrogenated soy oil (Dritex S, AC Humko, Memphis, TN) with a melting point of about 80°C or 140-160°F was sprayed on the creatine/arginine powder as it was mixing in the Littleford mixing unit. Efficient coating or microencapsulation of the powder was achieved in about 5 minutes when a temperature of about 155°F was reached and the hot oil thoroughly mixed with the powder. The plow amps were about 75-100. The resulting granules were small, free flowing, and exhibited sustained-release properties when a dissolution test was conducted. The weight percent of the finished product was 80% arginine/creatine, 20% hydrogenated soy oil.

Dissolution Test

Protocol:

Basket method

Paddle speed: 50 RPM

Media: water

Time points: 1,2,4, and 6 hours

Results: % release

	<u>Creatine</u>	<u>Arginine</u>
1 hr	46.5%	30.3%
2 hrs	63.6%	37.5%
4 hrs	78.6%	42.8%
6 hrs	84.9%	46.1%

Differences in solubility between the arginine and the creatine account for the different release profiles, but both substances were clearly sustained-release.

The formulation of example 2 was then blended with suspending agents, sweetener, and flavor to yield a pleasant tasting powder that could be mixed with water or juice to yield a dose of 5 grams of creatine monohydrate and 5 grams of L-arginine free base. This formulation produced a sustained-release delivery system designed to increase the storage and delivery of creatine to the muscles, while simultaneously increasing vasodilation or blood flow to the muscles and enhancing aerobic capacity.

Example 3

Creatine monohydrate and L-arginine free base are added in equal amounts to a Hobart type mixer that is jacketed with a circulating hot water bath which is capable of operating at high temperatures. A hydrogenated soy oil (Dritex® S, AC Humko, Memphis, TN) with a melting point of about 80°C or 140-160°F was mixed with the creatine/arginine powder at a

temperature of about 180°F until complete melting of the oil. Efficient coating or microencapsulation of the powder was achieved in about 5-10 minutes when the hot oil thoroughly mixed with the powder. The mixer was then cooled by running cool water through the jacketed system surrounding the unit, and an ethylcellulose solution (Surelease®, Colorcon, Westpoint, PA) at 25% solids in solution was applied by sprayer. The resulting granules were small, free flowing, and exhibited sustained-release properties when a dissolution test was conducted. The weight percent of the finished product was 75% arginine/creatine, 20% hydrogenated soy oil, and 5% ethylcellulose.

Dissolution Test

Protocol:

Basket method

Paddle speed: 50 RPM

Media: water

Time points: 1,2,4,6 , and 8 hours

Results: % release

L-Arginine free base

1 hr 20%

2 hrs 30%

4 hrs 40%

6 hrs 46%

8 hrs 52.4%

Example 4

The formulation of example 2 will be tested in athletes and body builders and shown to increase muscle mass and contribute to sustained or prolonged endurance. This formulation will be shown to be particularly

effective in sports activities of long duration. In addition, the formulation of example 2 will be shown to significantly enhance or increase creatine transport or creatine loading when compared with creatine alone. This is an unexpected result, which has heretofore not been suggested or demonstrated in animals or humans as far as the inventor is aware.

WHAT IS CLAIMED IS:

1. An oral exercise enhancing composition of creatine and an insulin mediating substance that induces vasodilation.
2. A composition of claim 1 wherein the insulin mediating substance is L-arginine, L-citrulline, L-lysine, L-ornithine, L-glutamine, thier salts, complexes, esters, or peptides and the creatine is creatine monhydrate.
3. A composition of claim 1 wherein the amount of creatine is at least 1 gram
4. A composition of claim 1 wherein the insulin mediating substance is sufficient to elicit a significant increase in blood flow to the extremities and muscles.
5. A composition of claim 1 wherein both or one or the other substances is in sustained-release form.
6. A composition of claim 5 wherein the sustained-release agent comprises algal polysaccharides, chitosan, pectin, glucomannan, guar gum, xanthan gum, gum arabic, gum karaya, locust bean gum, keratin, laminaran, carrageenan, cellulose, modified cellulosic substances such as cellulose ether derivatives; methylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, hydroxyethylcellulose, sodiumcarboxymethylcellulose, carboxymethylcellulose carboxypolymethylene, acrylic resin polymers, polyacrylic acid and homologues, polyethylene glycol, polyethylene oxide, polyhydroxylalkyl methacrylate, polyvinylpyrrolidone, polyacrylamide, agar, zein, stearic acid, high melting point oils, waxes, gelatin, or combinations thereof.

7. A composition of claim 6 wherein the sustained-release particles are produced by coating with an oil with a melting point greater than 100°F.
8. A composition of claim 7 wherein the oil is a hydrogenated soy oil with a melting point of approximately 150°F.
9. A composition of claim 2 wherein the amount of creatine monohydrate in a single dose is about 500 mg. to 10 grams, and the insulin mediating agent is L-arginine.
10. A composition of claim 8 wherein the amount of L-arginine in a single dose is 2-30 grams.
11. A composition of claim 8 wherein the amount of creatine monohydrate in a single dose is 5 grams and the amount of L-arginine in a single dose is 5 grams
12. A composition of claim 5 wherein the creatine monohydrate and the L-arginine are sustained-release.
13. A composition of claim 12 wherein the amount of sustained-release L-arginine is 5 grams and the amount of sustained-release creatine monohydrate is 3 grams.
14. A method of enhancing athletic performance and exercise capacity in a mammal by coadministration of creatine with an insulin modulating agent that increases vasodilation.
15. The method of claim 14 wherein the insulin modulating agent is L-arginine, L-citrulline, L-ornithine, L-lysine, L-glutamine, thier salts, esters, peptides, or complexes with other agents.

16. The method of claim 14 wherein both substances are in sustained-release form.
17. The method of claim 14 wherein the insulin modulation agent is L-arginine and the creatine is creatine monohydrate.
18. The method of claim 16 wherein the composition comprises L-arginine free base and creatine monohydrate in sustained-release form.
19. The method of claim 14 wherein a single dose is 1-30 grams of each substance.
20. The method according to claim 19 wherein the amount of creatine is 2-5 grams per dose and the insuline modulating agent is L-arginine in a dose of 3-8 grams per dose.
21. The method of claim 20 wherein the creatine is sustained-release and in an amount of 3-5 grams and the L-arginine is sustained-release in an amount of 3-6 grams in a single dose.
22. A method of increasing muscle mass in body builders or weight lifters by coadministering creatine and arginine or its biological equivalents.
23. The method of claim 22 wherein the arginine is in sustained-release form and the creatine is in immediate-release form.
24. The method of claim 22 where a single dose of creatine is about 1-10 grams and the single dose of arginine is 1-30 grams.
25. The method of claim 22 where the single dose of creatine is 3-5 grams and the single dose of arginine is 2-6 grams.

26. The method of claim 22 wherein both arginine and creatine are in sustained-release form.
27. The method of claim 26 where the dose of arginine is 1-30 grams and the dose of creatine is 1-10 grams.
28. The method of claim 27 where the dose of arginine is 5 grams and the dose of creatine is 5 grams.
29. The method of claim 22 wherein the biological equivalent is L-citrulline, L-lysine, L-ornithine, or L-glutamine, thier salts, esters, peptides, or dipeptides.
30. A method of enhancing creatine transport and storage in muscles by the coadministration of creatine with an insulin modulating agent such as arginine.
31. The method of claim 30 wherein the arginine is in sustained-release form.
32. The method of claim 30 where the creatine and the arginine are both in sustained-release form.